

U.S.S.N. 09/779,957
Filed: February 9, 2001
RESPONSE AND AMENDMENT

In the Claims

1. (currently amended) A DNA construct for expression of multiple gene products in a cell comprising:

- (a) a single promoter at the 5' end of the construct,
- (b) an intein splicing unit comprising multiple genes, or exons, two or more extein sequences encoding one or more proteins, and
- (c) one or more intein sequences, fused to the carboxy-terminus encoding portion of each gene extein sequence, except the last gene extein sequence to be expressed, and
- (d)(e) a 3' termination sequence comprising a polyadenylation signal following the last extein protein coding sequence,

wherein the intein splicing unit is expressed as a precursor protein containing at least one intein flanked by extein encoded proteins; and wherein at least one of the intein inteins sequences can catalyze excision of the exteins, and wherein the excised exteins are not ligated.

Claim 2-5. (canceled)

6. (original) The construct of claim 1 wherein the promoter is selected from the group consisting of inducible promoters, constitutive promoters and tissue specific promoters.

7. (currently amended) The construct of claim 1 wherein the genes extein sequences encoding one or more proteins are preceded or followed by a sequence encoding a peptide that targets the gene expression product to a particular compartment within the cell in which the construct is expressed.

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8. (original) The construct of claim 1 wherein the proteins are different enzymes.
9. (original) The construct of claim 1 wherein the proteins are the same proteins.
10. (currently amended) The construct of claim 1 wherein the inteins intein splicing unit prevent expression product prevents the ligation reactions normally associated with protein splicing.
11. (previously presented) The DNA construct of claim 10 wherein the DNA construct encodes a glycine or alanine linking the intein and extein amino acid sequences.
12. (previously presented) The construct of claim 29 wherein the proteins are selected from the group consisting of acyl CoA dehydrogenases, acyl CoA oxidases, catalases, alpha subunits of beta-oxidation, beta subunits of beta-oxidation, PHA synthases with medium chain length substrate specificity, beta-ketothiolases, NADH or NADPH dependent reductases, PHA synthases with short chain length specificity, and PHA synthases that incorporate both short and medium chain length substrates.
13. (previously presented) The construct of claim 29 wherein the proteins are selected from the group consisting of enzymes encoded by the phuG locus, medium chain length synthases, beta-ketothiolases, NADH or NADPH dependent reductases, and PHA synthases that incorporate both short and medium chain length substrates.
14. (previously presented) The construct of claim 29 wherein the proteins are selected from the group consisting of herbicide resistance, insect resistance, and marker proteins.

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15. (currently amended) A method for expressing multiple genes in cells comprising transforming the cells with a DNA construct comprising:

- (a) a single promoter at the 5' end of the construct,
- (b) an intein splicing unit comprising multiple genes, or exteins, two or more extein sequences encoding one or more proteins, and
- (c) one or more intein sequences, fused to the carboxy-terminus encoding portion of each gene extein sequence, except the last gene extein sequence to be expressed, and
- (d) (c) a 3' termination sequence comprising a polyadenylation signal following the last coding sequence;

wherein the intein splicing unit is expressed as a precursor protein containing at least one intein flanked by extein encoded proteins; and wherein at least one of the intein inteins sequences can catalyze excision of the exteins, and wherein the excised exteins are not ligated.

Claims 16-17. (canceled)

18. (original) The method of claim 15 wherein the cell is a plant cell and the promoter is a promoter operable in a plant cell.

Claim 19. (cancelled)

20. (original) The method of claim 15 wherein the promoter is selected from the group consisting of inducible promoters, constitutive promoters and tissue specific promoters.

21. (currently amended) The method of claim 15 wherein the genes extein sequences encoding one or more proteins are preceded or followed by a sequence encoding a peptide that

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targets the gene expression product to a particular compartment within the cell in which the construct is expressed.

22. (original) The method of claim 15 wherein the proteins are different enzymes.

23. (original) The method of claim 15 wherein the proteins are the same proteins.

24. (currently amended) The method of claim 15 wherein the intens intein splicing unit prevents prevent the ligation reactions normally associated with protein splicing.

25. (previously presented) The method of claim 24 wherein the DNA construct encodes a glycine or alanine linking the intein and extein amino acid sequences.

26. (previously presented) The method of claim 18 for making polyhydroxyalkanoates in plants wherein the proteins are selected from the group consisting of acyl CoA dehydrogenases, acyl CoA oxidases, catalases, alpha subunits of beta-oxidation, beta subunits of beta-oxidation, PHA synthases with medium chain length substrate specificity, beta-ketothiolases, NADH or NADPH dependent reductases, PHA synthases with short chain length specificity, and PHA synthases that incorporate both short and medium chain length substrates.

27. (original) The method of claim 18 for making polyhydroxyalkanoates in plants wherein the proteins are selected from the group consisting of enzymes encoded by the phaG locus, medium chain length synthases, beta-ketothiolases, NADH or NADPH dependent reductases, and PHA synthases that incorporate both short and medium chain length substrates.

28. (previously presented) The method of claim 18 wherein the proteins are selected from the group consisting of herbicide resistance, insect resistance, and marker proteins.